Re-epithelialization: advancing epithelium frontier during wound healing

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The first function of the skin is to serve as a protective barrier against the environment. Its loss of integrity as a result of injury or illness may lead to a major disability and the first goal of healing is wound closure involving many biological processes for repair and tissue regeneration. In vivo wound healing has four phases, one of them being the migration of the healthy epithelium surrounding the wound in the direction of the injury in order to cover it. Here, we present a theoretical model of the re-epithelialization phase driven by chemotaxis for a circular wound. This model takes into account the diffusion of chemoattractants both in the wound and the neighbouring tissue, the uptake of these molecules by the surface receptors of epithelial cells, the migration of the neighbour epithelium, the tension and proliferation at the wound border. Using a simple Darcy’s law for cell migration transforms our biological model into a free-boundary problem, which is analysed in the simplified circular geometry leading to explicit solutions for the closure and making stability analysis possible. It turns out that for realistic wound sizes of the order of centimetres and from experimental data, the re-epithelialization is always an unstable process and the perfect circle cannot be observed, a result confirmed by fully nonlinear simulations and in agreement with experimental observations.

1. Introduction

Adult skin is made up of three layers: the epidermis and the dermis separated by the basement membrane. When a profound injury occurs which destroys a part of the dermis, it has to be mended rapidly to restore the protective barrier [1,2]. In vivo wound healing is a complex repairing process orchestrated by intra- and intercellular pathways. To recover the integrity of the skin, this process is accomplished by four successive but overlapping stages: clotting, inflammation, re-epithelialization and remodelling. Immediately after an injury, a clot composed mainly by fibrin fibres and platelets is formed to plug the wound (stage 1). Subsequently in the next 2–10 days (stage 2) the clot is continuously infiltrated by inflammatory cells which clear up the debris and release chemical factors, such as vascular endothelial growth factor and transforming growth factor-beta. Once the chemical gradient is established, different cells are recruited to fabricate the new tissue (stage 3). On the one hand, neo-vasculature [3] and fibroblasts (depositing collagen) are recruited in the dermis, reforming the clot into a granulation tissue, which nutritiously and physically support the repair of upper layers. On the other hand, keratinocytes migrate and proliferate at the edge of the wound to extend the newly formed epithelial carpet made of several layers of cells in the epidermis. This process is called re-epithelialization and it lasts for two to three weeks. At the end of stage 3, myofibroblasts transformed from fibroblasts contract and try to bring the wound edge together. They disappear by apoptosis in the dermis. The remodelling (stage 4) continues for months or even for years in order to restore the homoeostasis of the normal skin. However, the normal anatomical structure is not truly recovered and a
and a 6 mm diameter punch biopsy on an artificial corneal tissue (in vitro) driven by morphogens in the granulation tissue [9]. Very membrane, migrate towards the wound margin and proliferate, the epidermal predominant cells, detach from the basal membrane, during the four stages.

The closing process after a 5 mm diameter punch biopsy on the normal skin of wild-type mice (a(i), from [14], copyright with permission) and a 6 mm diameter punch biopsy on an artificial corneal tissue (b(ii), from [15], copyright with permission). Both present a wavy border as the wound closes. The photographs taken 3 days (for the wild-type mice skin) and 2 days (for the corneal tissue) after the punch are shown in a(ii) and b(ii), respectively.

Here, we focus on re-epithelialization when keratinocytes, the epidermal predominant cells, detach from the basal membrane, migrate towards the wound margin and proliferate, driven by morphogens in the granulation tissue [9]. Very careful in vitro epithelium migration experiments have been performed recently by several groups [10–13]. Realized on a solid substrate, they involve an advancing monolayer motivated by the collective behaviour of cells through migration, generation of forces and reorganization of the cell cytoskeleton at the margin. The findings indicate a non-intuitive manner of cells to achieve robustness during the repair process. After a few days, the wound fills with the advancing epithelial layer. The finding indicates a non-intuitive manner of cells to achieve robustness during the repair process.

2. The model

We consider a circular hole \( \Omega \) in a two-dimensional cellular population of density \( \rho \) immersed in a morphogenetic environment (figure 2). Cells migrate up the morphogen gradient, the chemotactic flux being proportional to the concentration gradient of morphogens \( F = \lambda_c \nabla c \), with \( \lambda_c \) a mobility constant. The morphogen repellent \( \frac{\partial \phi}{\partial r} \) inside and outside the hole \( \Omega \) is alimented through sources coming from below. Mitosis happens only at the border [15,31] and the closure is mostly achieved by the migration. The mass balance for the cell population is then

\[
\rho + \nabla \cdot (\rho v) = \gamma \rho + \nabla \cdot F. \quad (2.1)
\]

Neglecting volumetric growth (\( \gamma = 0 \)) in the tissue except at the periphery, the cell density is constant and \( \rho = \rho_0 \). Equation (2.1)
simplifies to $\nabla(p\nu - A_c\nabla c) = 0$ and gives that the normal velocity of the front is directly proportional to the normal concentration gradient. At extremely low velocities, the cell migration satisfies a Darcy’s law $\nu = -M_p \nabla p$, $M_p$ being a porosity coefficient equal to the square of the epithelium height divided by a friction coefficient. As shown in [18], this law is deduced in tissues when friction between phases or friction with a substrate balances the hydrostatic part of the elastic stress acting on the cells [18,20]. The wound perturbs the homostatic state of the surrounding and a source of morphogens is the outer normal. $\partial W$.

\[ \Delta(p + A_c) = 0 \] with the index $h$ or $e$ referring to the inner (hole) or outer (epithelium) domain. $\delta = D_h/D_e$ represents the ratio between the diffusion coefficient in the hole $D_h$ and the tissue $D_e$, it is bigger than 1, and $\alpha$ gives the strength of the transverse flux which maintains the morphogen level inside the hole. Owing to the relative slowness of the cell migration, we neglect the time dependence in equation (2.2). Taking $D_e/M_p$ as pressure unit simplifies Darcy’s law into $\nu = -\nabla p$, where for simplicity we keep the same notation for $p$ and $\nu$. From mass balance equation (2.1), we get the following Laplace equation coupling the unknown pressure $p$ to the chemoattractant concentration $c$:

\[ \Delta(p + A_c) = 0 \]

So, we get $p = -A_c + \phi$, where $\phi$ is a holomorphic function which satisfies $\Delta \phi = 0$. Finally, we must pay special attention to the interface boundary conditions. For equation (2.2), they concern the concentration continuity and the flux discontinuity owing to the morphogen consumption by mitosis at the border $\partial \Omega$ (for demonstration, see [18]):

\[ c_h = c_e \quad \text{and} \quad \Delta N \nabla c_h = N \nabla c_e + \Gamma_2 c_h. \]

where $N$ is the outer normal. $\Gamma_2$ is the uptake rate constrained at the border discussed below together with the mitosis rates $\Gamma_1$ and $\Gamma_1'$ at the border. Capillarity fixes the pressure jump at the interface

\[ p = p_0 - \sigma \frac{1}{R}, \]

which gives the free-boundary condition considering the geometric effect where $R$ is the local radius of curvature. $R$ is equal to the radius if the geometry is a circle. However, it considers the local effect at small length scale when the boundary is perturbed. At the same time, the velocity $dR/dt$ of the interface differs from that of the epithelium $v$ by the cell proliferation at the border and we get

\[ V_{int} = N \frac{dR}{dt} = N \nu - \Gamma_1 c. \]

\[ \Gamma_1 \]

where $\Gamma_1$ is the mitosis rate and $\sigma$ the capillary number related to the tension $T$ at the interface so $\sigma = (T \cdot M_p)/(D_0^2 \tau^2)$.

Wound healing in vivo is dominated by two-dimensional migration and the morphogens are probably not the nutrients, so $\Gamma_2$ vanishes and $\Gamma_1 c$ must be replaced by $\Gamma_1'$ if we also consider mitosis without the quantitative effect of the nutrient concentrations. The capillary number $\sigma$ may involve the activities of actin cable (bundled microfilaments) in the cells. During embryonic wound healing, the leading edge cells can coordinate their actin cables globally which generate an effect on the macroscopic level. However, this coordinated behaviour is lost in adults, so the effect of actin cables may act only locally. This resembles $\sigma$ in our model which is not effective for large wounds. Considering the set of equations (2.2) and (2.3) in conjunction with the set of boundary conditions (2.4), (2.5) and (2.6) shows that chemoattract-driven migration is indeed a free-boundary problem [32,33] involving several parameters to represent the biological complexity and the study of simple cases, for example the circular closure, may help in understanding their corresponding roles. Thus, we present first the analytical results for a hole remaining circular at all times, then its stability.

3. The results

3.1. Regular circular closure

In the quasi-static approximation, equation (2.2) can be solved analytically and gives

\[ C_0 = \begin{cases} 
1 + \frac{(A - 1)I_0(ar)}{I_0(aR)} & \text{if } r \leq R \\
\frac{AK_0(r)}{K_0(R)} & \text{if } r > R.
\end{cases} \]

$I_0$ (resp. $K_0$) being the modified Bessel function of zero order, regular at $r = 0$ (resp. $r \to \infty$). Equation (3.1) takes into account the continuity at the interface, $A$ being given by the flux continuity and reads

\[ A = \frac{(a\delta)K_1(R)}{[a\delta]K_1(R) + I_1(aR)],} \]

with the following definition for $I_s(X) = I_{s-1}(X)/I_s(X)$ (ratio of two successive Bessel functions) and the equivalent for $K_s(X) = K_{s-1}(X)/K_s(X)$. The pressure $P_0$ inside the epithelium becomes

\[ P_0(r,t) = -\Pi A \left( \frac{K_0(r)}{K_0(R)} - 1 \right) + A_0 \log \left( \frac{R}{R} - \frac{\sigma}{R} \right). \]
We use the Laplace law to fix the unknown degree of freedom. The holomorphic function \( \phi \), proportional to \( \log(r) \), represents a possible driving force which appears at the interface (i.e., the so-called kenotaxis defined in [13]). Indeed, even in the absence of morphogens, in vitro epithelium migration on solid substrate is observed, perhaps owing to the reorganization of the cellular cytoskeleton at the wound margin. Note that the model remains valid if we discard the chemotaxis provided the definition of length and time units is modified. Furthermore, \( C_0 \) and \( P_0 \) are dependent on time via \( \mathcal{R}(t) \). For simplicity, we drop the time dependence of \( R \). The velocity \( \mathcal{R} \) of the closure deduced from equation (3.5) is then

\[
\mathcal{R} = -\frac{(A + \Gamma_1) A}{K_1(R)} - \frac{A_{in}}{R}.
\]  

(3.4)

The closing velocity is constant for large holes \( \mathcal{R} = -\alpha \delta (A + \Gamma_1)/(1 + \alpha \delta) \) and is exponentially small \( \mathcal{R} \sim e^{-\alpha \delta (A + \Gamma_1)/(1 + \alpha \delta)} \) when the radius \( R \) becomes tiny, if one restricts on chemo- tactic migration and proliferation. So the radius of a large hole begins with a linear decrease in time but the total closure will take an infinite time for a complete achievement. For small holes, chemotaxis becomes subdominant compared with kenotaxis \( A_{in} \) at the interface which controls the closure dynamics and the radius satisfies a diffusive law in \( t^{1/2} \) [12]. Figure 3 shows \( \mathcal{R} \) as a function of \( R \) for different values of \( \alpha \), \( \delta \) and \( A_{in}/A \) and §4 contains a discussion of parameters.

3.2. Loss of circularity

However, equation (3.4) is valid only if the circular contour is maintained. It is why we perform a linear stability analysis for large wounds (\( A_{in} \sim 0 \)) assuming a small harmonic perturbation for the radius as \( R_0(t) = R(t)[1 + e^{-\epsilon \lambda_1(t)} \cos(n\theta)] \) inducing variations on the pressure \( P \) and concentration field \( C \) of the same order \( \epsilon \) as follows:

\[
C(r, \theta, t) = C_0(r, t) + \epsilon C_i(r, t) e^{i \lambda_1 \cos(n\theta)}
\]

\[
P(r, \theta, t) = P_0(r, t) + \epsilon P_i(r, t) e^{i \lambda_1 \cos(n\theta)},
\]

where the subscript \( i \) indicates either a quantity relative to the hole (\( h, 0 < r < R \)) or to the epidermis (\( e, R < r < \infty \)). Although all perturbative quantities depend on the selected mode \( n \), the linear perturbation analysis treats these modes independently. So we drop the \( n \) index and calculate the perturbative concentration fields \( c_i(r) \) from equation (2.3)

\[
c_i = \begin{cases} \chi_0 I_n(\alpha R) & \text{with } \alpha_1 = \sqrt{\alpha^2 + \Omega_0/\delta} \\ \chi_0 \frac{K_n(\alpha R)}{K_0(\alpha_2 R)} & \text{with } \alpha_2 = \sqrt{1 + \Omega_0}, \end{cases}
\]

(3.6)

while the perturbed pressure field is given by \( p_i(r) = BR^n/r^n - \mathcal{R} c_i(r) \) where we take into account the harmonic modes of the holomorphic function \( \phi \) with \( B \) fixed by the Laplace law \( B = RR + \sigma(n^2 - 1)/R \). Owing to the weakness of \( \epsilon \), our system of equations, once linearized, can be solved analytically and the growth rate of the mode \( n \) reads

\[
\Omega_n = -\sqrt{n(n^2 - 1)/R^3} + \mathcal{R} [n - 2 - R K_1(R)] - c_n R K_n(\alpha_2 R),
\]

(3.7)

where

\[
c_n = \alpha_2 \left( \alpha_1 K_1(R) + (\delta - 1)(\alpha_1 I_n(\alpha_1 R) - n/R) \frac{(\delta - 1)n^2 - 3R^2}{R^5} + \frac{\alpha_1^2 K_0(\alpha_1 R)}{K_0(\alpha_2 R)} \right).
\]

Equation (3.7) represents an implicit relationship for \( \Omega_n \) solved by iterative techniques and positive values indicate the modes responsible for the destabilization of the circular border as the migration proceeds. The results are presented for different values of \( A, \alpha \) and \( \delta \), where \( \Omega_n \) is displayed in figure 4. See §4 for the discussion of parameters. The results indicate an instability leading to a deviation from a circle in short time, for \( n \) up to a critical mode \( n_c \) (fixed by the capillarity). It is why numerical simulations are necessary to go beyond the linear analysis and fully consider the nonlinearities.

3.3. Full dynamics and numerical methods

We discretize \( c \) in equation (2.2) and pressure \( p \) in equation (2.3) on a Cartesian mesh in space and implicitly in time, using a nonlinear adaptive Gauss–Seidel iterative method [29,30]. At the domain boundary, we impose both vanishing values of \( c \) and normal gradient of \( p \). Then the noise is added on \( C = c + \chi \) with \( |\chi| \ll 1 \). Beginning with a perfect circle with \( R = 90 \), the wound closing is tracked by the level-set method developed in [27,28], where a scalar function \( \Phi \) with \( \nabla \Phi = 1 \) describes the wound (\( \Phi < 0 \)), the epithelium (\( \Phi > 0 \)) and the interface (\( \Phi = 0 \)). The normal and curvature

![Figure 3. The closing velocity \( \mathcal{R} \) as a function of \( R \), varying (a) \( \delta \), (b) \( \alpha \) and (c) \( Q = A_{in}/A \). For large \( R \), the velocity is constant. The velocity converges to 0 when \( R \) goes to 0 except that the effect of \( A_{in}(>0) \) is considered (c).](image-url)
4. Discussion and conclusion

There are several independent parameters in this model. We can fix some of them with the published experimental data (table 1). Our velocity unit is compatible with the closing velocity of the cornea (3 days for a hole of radius approximately 3 mm [15]) giving \( \Lambda \approx 1 \) for this experiment according to equation (3.2). In figure 4, we vary the parameters \( \alpha \) and \( \delta \), which are more difficult to estimate, and also \( \Lambda \). Owing to the large size of wounds in practice, the linear stability analysis gives always an instability and this conclusion is robust to parameter changes. This finding is confirmed by fully nonlinear simulations under the same parameter range (figures 5 and 6). In the simulations, large \( \Lambda \) (approx. 10, total time approx. 600) contributes to faster closing compared with small \( \Lambda \) (approx. 1, total time approx. 600). A typical example of the closing process with \( \alpha = 1 \) and \( \delta = 2 \) is shown in figure 5a. The advancing interface becomes wavy as the wound heals; however, when the wound becomes small, surface tension re-stabilizes the wound to a potato shape consistent with the linear stability analysis. This surface tension may also pinch wounds off to smaller pieces as shown in figure 5b(ii), figure 6 and in [12]. This event requires at first a more irregular closing (red arrows in figure 6), when the concentration of chemoattractant is less homogeneous given inadequate transverse flux (\( \alpha = 0.1 \)). The pinch-off can happen both at the intermediate or the end stage of the closing (yellow arrows), consistent with observations at the late stage of wound healing on a monolayer of MDCK cells (figure 6, right), suggesting the same behaviour in more complex wound-healing processes.

In this work, we have shown theoretically that wound re-epithelialization gives a border instability under clinically realistic parameters. Driven by chemotaxis, our model does not introduce other cell activities which close the wound at the ultimate stage. However, we conjecture that this chemotactic instability at the border of the wound may affect the quality of the final repair. During embryonic wound healing where perfect reconstruction is observed, the wound is closed
Table 1. The parameter table.

<table>
<thead>
<tr>
<th>physical parameter</th>
<th>value</th>
</tr>
</thead>
<tbody>
<tr>
<td>diffusion coefficient $D_c$</td>
<td>$1\ \mu m^2\ s^{-1}$ [34]</td>
</tr>
<tr>
<td>uptake time $\tau_u$</td>
<td>2000 s [34]</td>
</tr>
<tr>
<td>surface tension $T$</td>
<td>$10^{-4}\ N\ m^{-1}$ [12]</td>
</tr>
<tr>
<td>friction coefficient $\alpha$</td>
<td>$10^{-5}\ N\ \mu m^{-1}$ [12]</td>
</tr>
<tr>
<td>the length unit $m$</td>
<td>50 $\mu m$, calculated</td>
</tr>
<tr>
<td>the velocity unit $m/s$</td>
<td>$0.025\ \mu m\ s^{-1}$, calculated</td>
</tr>
<tr>
<td>the capillary number $\sigma$</td>
<td>0.1, calculated</td>
</tr>
<tr>
<td>the chemoattractant strength $\alpha$</td>
<td>0.1–10, estimated</td>
</tr>
<tr>
<td>the diffusion coefficient ratio $\delta$</td>
<td>0.1–10, estimated</td>
</tr>
<tr>
<td>the cell velocity $A$</td>
<td>1–10, estimated</td>
</tr>
</tbody>
</table>

by a ‘purse string’ which represents a coordination of leading edge cells facilitated by the actin cable [35]. This mechanism is lost in the adult skin, where the wound is closed by the crawling of cells (several layers) on the granulation tissue [36]. Compared with a static substrate in vitro, the granulation tissue undergoes contraction at the end of the re-epithelialization by myofibroblasts [37]. Indeed, this purpose is to bring the edge together which resembles the ‘purse string’, but the contraction by those cells needs to be compatible with the synchronous material constitution, which is mechanically and systematically a challenge [7].

At the end, we discuss our model in the context of the skin wound healing in vivo. As the wound goes deeply into the dermis which is the case for most wounds, the two layers behave differently during re-epithelialization. The depth of a wound contributes to the thickness in the lower layers behave differently during re-epithelialization. The re-epithelialization where only several layers of cells become motile above the lower stacks where the chemoattractant is released, the depth of the wound contributes to the quality of the final repair. Before that, the irregularity of the wound border driven by chemotaxis should be considered as an ingredient.

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Appendix A. Numerical implementation

The simulation is done on a $200 \times 200$ Cartesian lattice with a grid size of 10 $\mu m$. This free-boundary problem is easy to be implemented with the level-set method owing to its dependence on the curvature in solving the pressure in equation (2.5). The full method is adapted from [28,29] and has an order of accuracy of more than 1.5. While the boundary can be implicitly given by the zero contour of the level-set function $\Phi$, the curvature can also be readily calculated. At each time step, the domain from $\Omega_t$ to $\Omega_{t+\Delta t}$ is updated as follows:

- Solve equation (2.2) with the fixed domain $\Omega_t$ in the steady state with the free-boundary condition equation (2.4). At the boundary of the computational lattice, the Neumann boundary condition is imposed.
- Update the solution of concentration $C$ by $C = c + \chi$ pointwisely, where $\chi$ is randomly generated out of uniform distribution between $-|\chi_c|$ and $|\chi_c| \cdot |x_c| = 0.08$ is used for the presented simulations.
- Solve equation (2.3) with known $C$ under the interfacial boundary condition from the first part of equation (2.5), where the local curvature $1/R$ is calculated from the level-set function by $\nabla \cdot (\nabla \Phi / |\nabla \Phi|)$. The Neumann boundary condition is imposed at the boundary of the computational lattice.
- Calculate the velocity $V$ of the moving boundary from the second part of equation (2.4), where $N$ is calculated from the level-set function by $\nabla \Phi / |\nabla \Phi|$. 
- Find the appropriate $\Delta t$ given by the CFL condition by $\Delta t \leq \Delta x/4\max |V|$ and update the domain $\Omega_t$ to $\Omega_{t+\Delta t}$.
- Start with the newly updated domain $\Omega_{t+\Delta t}$ and repeat the last five steps.

References


